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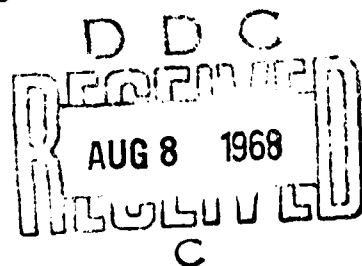
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UNITED STATES ARMY  
CHEMICAL CORPS BIOLOGICAL LABORATORIES  
Fort Detrick, Maryland

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On Penetration of Anthrax Spores Through the Intact Pulmonary Surface  
of the Sheep.

by Dr. Eugen Enderlen.

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Translated from Deut. Z. f. Thiermedizin 15:50-56 (1889)  
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In the winter of 1887 to 1888 H. Buchner had made numerous experiments on the penetration of pathogenic bacteria through intact pulmonary surface, the results of which were published in the Archives for Hygiene, 1888, Volume 8. Anthrax spores were dried on coal dust or on spores of *Lycoperdon giganteum*. As a last step a suspension of anthrax spores in distilled water was atomized by means of the so-called indirect spray method. By the latter method infection of animals through the lung succeeded in 68% of the tests; it was possible, moreover, to demonstrate invasion of bacteria (anthrax rods) in sections. Animals used for inhalation included mice, guinea pigs and rabbits. It was indicated to subject larger animals to atmospheric infection with anthrax spores, since infected pastures certainly offer manifold occasions for their vaporization. Sheep, which are highly susceptible to anthrax, seem to be suited best for this purpose. Indirect spray was used in tests to be discussed later.

The latter was produced in a large double-neck Wulf's flask; the glass tubes were attached so that the spray did not take a horizontal direction but described a nearly vertical path. The flask was cut off below and placed on a tin vessel. A tight closure was made with a rubber band. Two rubber tubes passed through the neck of the flask; one serves for the admission of air from a bellows, the other for renewal of fluid. When the apparatus is started one can observe a fine fog appearing from the glass tube attached to the second neck which then is conducted into a kettle serving as an inhalation chamber <sup>1/</sup>. This fog carries the bacteria. One side of the kettle is equipped with an opening to which a tin sleeve is attached. A hose-like, dense cloth is attached to a lip on the tin sleeve and this is drawn over the animal's head and neck after placement in the inhalation chamber. Cotton is used to obtain good closure so that only adequately filtered air can escape. Inhalation was carried out in the open air; the apparatus was placed in such a manner that possibly escaping spores were carried off by the wind. After all of the fluid was atomized we waited a little longer so that spores could settle on the walls; the animal was taken out, the kettle and the atomizing apparatus was well disinfected and finally washed with well water.

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<sup>1/</sup> A precise depiction of the apparatus is found in Archiv fuer Hygiene, Vol. 8, page 193,

Several experiments made by Buchner had shown that  $\frac{1}{2}\%$  of the fluid atomized in the flask reaches the animal chamber. This amount was subsequently fed to a second animal in order to counter the objection that the inhalation animal had ingested the spores and succumbed to an infection of the intestinal canal. As a matter of fact the amount fed to the animal was always too great. Of the  $\frac{1}{2}\%$  which reached the inhalation chamber the lungs certainly did not absorb all; a portion was deposited on the kettle's walls and on the animal itself, another portion was swallowed, another retained by the external respiratory passages. In spite of this great amount we never saw an intestinal infection. The spores were dispensed in such a manner that  $\frac{1}{2}\%$  of the atomized suspension was thoroughly mixed with wheat bread and salt and fed in this form. The animals inevitably ate all of the offered mixture with good appetite. The fed animal may be used for inhalation in a subsequent test in order to exclude the possible assumption that it had become immune to anthrax through the first feeding.

In order to produce anthrax spores of maximal virulence we infected a well-nourished sheep subcutaneously with the lung of an anthracic mouse. The following day revealed no morbid manifestations; the animal expired after 48 hours.

Section. Well-nourished animal, marked meteorism, rigor mortis present. The site of injection shows no abnormal signs externally, the edges of the wound coalescing. During removal of the tissue we found only moderate hemorrhage. The abdominal walls are tightly stretched. Parts of the stomach immediately prolapsed into the opening during section. The distended stomach has displaced the transverse colon strongly downward. The pelvis contains a small amount of light serous fluid. The lungs are without adhesions, the pleural cavities are empty, the pericardium contains a small amount of serous fluid. The pulmonary pleura is normal, the tissues are soft, light, pink, containing normal air, blood and juices. In the bronchi there is some foam tinged with blood. The heart is normal. The spleen is somewhat swollen, the capsule is slightly distended, the sectional surface is reddish-dark brown, the structure is indistinct and the pulp is soft. Liver and kidneys have copious blood, their tissue is moderately soft. In the digestive tract there is very slight injection, no formation of carbuncles. Preparations of lungs, spleen, liver and kidneys yielded copious anthrax rods. Cultures of the spleen were made on both agar (FlWAg).

Test No. 1 (E<sub>1</sub>). Anthrax spores were scraped from a moderately wet agar tube with a wire loop and suspended in 100cc of distilled water. The fluid was only slightly turbid. This quantity was atomized within 30 minutes at 10:30 in the morning. The test animal was a fairly well-nourished sheep.

Feeding (Control to E<sub>1</sub>). Since 100cc were atomized, 8 drops of the suspension were fed. These were distributed on 2 parts wheat bread,

thoroughly mixed with salt and dispensed. The animal consumed everything with good appetite. During the following six days there were no morbid manifestations in either sheep; consumption of food was good. On the 7th day after initiation of the test the inhalation animal was found dead in the cage during the morning; the fed sheep remained entirely healthy.

Section. Good state of nourishment, slight meteorism, the right lung has a normal volume, the pleura is smooth. The major part of the superior lobe is strongly hyperemic, and feels somewhat more resistant than normal tissue. The lower portion, about 2 fingers wide, has a normal blood content. Volume of air is somewhat reduced in the hemorrhagic sites, otherwise normal air, juice and blood content. Bronchi contained foam tinged with blood. The left lung is normal and so is the heart. The spleen is barely enlarged, tissues have copious blood and are frangible. The liver and kidneys are also rich in blood and are somewhat soft. The stomach is slightly distended by gasses, the serosa is normal. Only very slight injection in the intestinal canal.

Organ preparations yielded moderate quantities of anthrax rods. New cultures were initiated on broth agar. The fed animal remained without morbid manifestations. After 3 weeks the test was considered terminated, since later infection from the intestinal tract could not be expected. In order to meet even this possibility the same animal was once again used for feeding.

Test 2 (E2). Anthrax spores were scraped from a moderately wet agar tube and suspended in 100cc of distilled water. The slightly turbid fluid was atomized within 25 minutes in the morning by means of indirect spraying.

Control of E2. Eight (8) drops of the suspension were placed on wheat bread and fed to the above-mentioned control animal (test 1). On the following day both sheep are lively, as they are also on the second. On the third day the inhalation animal lies completely apathetic in the cage. After about 96 hours (counting from the test) it is moribund. The fed sheep is healthy.

Section. Well-nourished body, a little meteorism, rigor mortis has disappeared. Nostrils and lips covered with sanguinous foam. The lungs are slightly retracted, no adhesions; in both pleural cavities some serous, slightly hemorrhagically stained fluid. Both lungs have normal volume, the pleura is smooth. In both inferior lobes there is a slight excess of blood. In the apex of the right superior lobe there is a hemorrhagic site about the size of a 50¢ piece, which feels somewhat more resistant than the surrounding tissue. Otherwise the content of juice, blood and air is normal; there is some foam tinged with blood in the bronchi. The heart is normal, the spleen is barely swollen but very congested and soft. The liver, kidneys and digestive tract are normal. All organs contain anthrax rods.

As already indicated the control animal showed no morbid signs. The test was considered terminated after 5 weeks. The objection may be raised that the animal had obtained immunity against anthrax through the first feeding; for this reason it was used for inhalation in the following tests.

Test 3 (E<sub>3</sub>). Preparation of the atomizing fluid in the customary manner; modification involves procurement of only 2/3 of the spores from a moderately wet agar tube; the suspension was only slightly turbid. One hundred (100) cc were atomized in the afternoon within 25 minutes. A new sheep was fed 8 drops. After 36 hours the inhalation animal showed the first morbid manifestations; after 48 hours it was completely apathetic, it breathed at long intervals, exitus after about 55 hours.

Section. Well-developed animal, nostrils and lips covered with sanguinous foam; no meteorism; no accumulation of fluid in the abdominal cavity, lungs barely retracted, pericardium depleted of fat. Both lungs are unaffected, the pleural cavities are empty, very little content in the pericardium. Pleurae are not turbid, tissues are soft, they have a light pink glow throughout; only at one point of the right interior lobe, approximately in the middle, there is a dark red spot about the size of a lentil. The lobes are soft under the knife and crackle; the sectional surface is light pink and contains a normal volume of air, blood and juice; the resistance is not increased anywhere. There is sanguinous foam in the bronchi, there is some coagulated blood in the vessels. The heart is normal. The spleen is somewhat swollen and tears during removal, the pulp is reddish-black and mushy. The liver and kidneys have much blood and the tissue is slightly soft. The digestive tract's serosa is somewhat injected, there are no hemorrhages. The adventitia of the aorta shows many spots with hemorrhages of pinhead size. In the organs and in the bronchial foam there are anthrax bacilli. The control sheep is healthy.

With the exception of E<sub>1</sub> the lungs were entirely unimpaired, aside from small hemorrhagic spots. One may ask what caused the hemorrhage in the right superior lobe. Considering the interval from inhalation to death, 7 days passed in E<sub>1</sub>, 4 days in E<sub>2</sub> and not quite 3 days in E<sub>3</sub>. There was enough time during the first test to form carbuncles. Professor Bollinger considers these hemorrhagic foci as carbuncle-like localizations.

I shall now discuss the results of microscopic examinations of sections taken from the lungs. The margin of hemorrhagic and healthy tissue was taken from the lung of the first inhalation animal (E<sub>1</sub>) and hardened in alcohol. Various stains according to Gram, Kuehne and Weigert gave the following results:

a. Infiltrated parts. Pulmonary epithelium without turbidness, nuclei are distinct. The contents in the alveoli is usually sparse; the latter consists partly of intact, partly of disintegrated red blood cells mixed with a few white blood cells. In addition there is very little

alveolar epithelium with distinct nuclei. Anthrax rods are found very rarely; isolated ones are located in capillaries; wherever several of them have accumulated, they are enclosed by capillary vessels; only a few are located in the tissue preparation, where they apparently were carried by the circulation.

b. Microscopically normal tissue. Alveolar epithelium is distinct, as are the nuclei, the alveoli contain nothing, there are relatively copious anthrax rods in the capillaries, sometimes arranged in pseudo-threads. The increased amount of bacilli apparently is due to the more favorable oxygen supply present at these sites.

E<sub>2</sub>. The tissue is generally normal, only very few alveoli contain a small number of red and white blood cells. There are numerous anthrax bacilli in the capillaries.

E<sub>3</sub>. The pulmonary tissue is normal, only a few alveoli (Fig. b) have a content as E<sub>1</sub>. In some places the capillary vessels are completely loaded with bacilli (compare Fig. a) which sometimes are arranged in pseudo-threads. The larger vessels are usually quite free of bacteria, they very rarely contain isolated ones. Cross sections of the aorta also yield rather large amounts of anthrax rods.

In the experiments conducted by H. Buchner, wet atomization produced 68% fatality and feeding 5%. In my tests all inhalation animals expired, the fed ones invariably survived. Although the number of inhalations is not large, the result allows the conclusion that positive success could also be expected from a larger series.

Now, since the above cases prove that inhalation of anthrax spores is much more dangerous than feeding (much more was ingested than could have reached the lung in the most favorable of cases), the conclusion is valid that some cases of spontaneous anthrax in grazing animals may be explained by inhalation <sup>1/</sup>. At any rate this assumption is valid if a section discloses neither intestinal foci nor cutaneous carbuncles. The animals thus create the conditions for vaporization in anthracic regions, either by the wet or the dry route.

Caption under the figures mentioned: (a) Section from an inhalation lung, anthrax rods in a capillary; (b) alveolus with red and white blood cells, the former at times in the process of disintegration.

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<sup>1/</sup> In the case of another infectious disease, i.e., tuberculosis, there is no doubt on the basis of morbid anatomical facts that the pathogenic fungus is capable of perforating intact tissue, e.g., the mucous membrane of the digestive tract or of the lung, followed by settlement and reproduction in certain organs of predilection (lymph nodes, bones, joints). (Compare Bollinger on the genesis and curability of tuberculosis. Ref: Nuench ned Wochen. Schr. 1888 No. 29, page 480.)